Lac REPRESSOR BINDING TO SINGLE-STRANDED POLYADENYLIC ACID

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1. Introduction

The regulation of transcription of the *lac* operon structural genes rests on complex formation between a protein, the *lac* repressor, and a region of DNA, the *lac* operator. The binding constant of the *lac* repressor for the *lac* operator is very high: K_0 10^{13} M^{-1} at 0.05 M ionic strength [1]. It has been shown by competition experiments using the membrane filtration technique [2,3] and by physico-chemical experiments [4,5] that the *lac* repressor can also bind non-specifically to non-operator DNA. The binding constant depends on the composition and on the structure of the nucleic acid and is several orders of magnitude lower than that of *lac* repressor–*lac* operator complex: $K_d \simeq 10^5$–10^8 M^{-1}. The binding of *lac* repressor to single stranded poly(dA) and poly(dC) has been studied by competition experiments with *lac* operator [3]. Poly(dC) did not show any competition at the concentration used whereas the binding constant for poly(dA) was one order of magnitude less than that of poly[d(AT)] which is the more efficient 'non-operator' binding DNA.

In this paper we report results on interactions between *lac* repressor and single-stranded polyadenylic acid (poly(A)) using circular dichroism. The conformation of the *lac* operator in the operator–repressor complex is not known. Therefore it was of interest to know whether *lac* repressor binds to single stranded nucleic acids and how the conformation of single strands is affected upon *lac* repressor binding. We chose poly(A) as a model because its conformation is very well known and is characterized by a large CD spectrum [6,7], and also because this polynucleotide has been used as a model compound in the study of nucleic acid–protein interactions. One could then try to correlate the effect of the *lac* repressor with that of known models such as oligopeptides [8–13].

2. Experimental

E. coli lac repressor (from strain BMH 493, a gift from Dr Müller-Hill) was purified by ammonium sulfate fractionation and column chromatography on phosphocellulose as described by Müller-Hill et al. [14]. This material was typically greater than 98% pure as judged by SDS–polyacrylamide gel electrophoresis [15]. A stock solution was prepared by dialyzing the protein against a buffer containing 0.2 M potassium phosphate and 0.1 mM dithioerythritol, pH 7.25. The protein concentration was measured by ultraviolet absorption using an extinction coefficient of 21 400 M^{-1} cm^{-1} per subunit at 280 nm [16].

Poly (A) was purchased from Miles. It was dissolved and dialyzed in a buffer containing 1 mM potassium phosphate and 0.1 mM DTE, pH 7.25. Small volumes of the concentrated repressor solution at high ionic strength were added to the polynucleotide sample. The ionic strength of the poly(A) changed from 1–5 mM during a binding experiment.

Circular dichroism spectra were recorded with a Jobin-Yvon Mark III apparatus at 25°C using 1 cm cells.

3. Results

In the wavelength range 250–300 nm *lac* repressor exhibits a small CD spectrum due to its aromatic chromophores (2 tryptophans, 8 tyrosines, 5 phenylalanines). Under our experimental conditions, its contribution to the observed spectrum of poly(A)–repres-

sor mixtures is very small as compared to that of poly(A) and can be neglected. Measurements were limited to wavelengths longer than 250 nm since below this wavelength the peptide chromophore makes the contribution of the *lac* repressor larger than that of poly(A) and the CD signal is difficult to interpret.

The CD spectrum of poly(A) exhibits a large positive band with a maximum at 262.5 nm. This spectrum is characteristic of the single strand ordered conformation of poly(A) [6,7].

Addition of *lac* repressor to poly(A) leads to an important decrease of the CD signal without any change in shape. As larger quantities of repressor are added one can notice a small shift of the maximum towards 265 nm (fig.1). The curve obtained by following the CD signal intensity at 265 nm does not reach a plateau in the concentration range that we have used (fig.2).

Addition of NaCl reverses the observed decrease of the CD spectrum of poly(A) (fig.1).

The decrease of the intensity of the CD signal of poly(A) is similar to that observed when the temperature is increased [6,7]. This change in the CD spectrum corresponds to a change of the polynucleotide conformation which clearly indicates that the *lac* repressor binds to the polynucleotide.

The fluorescence spectra of the *lac* repressor in

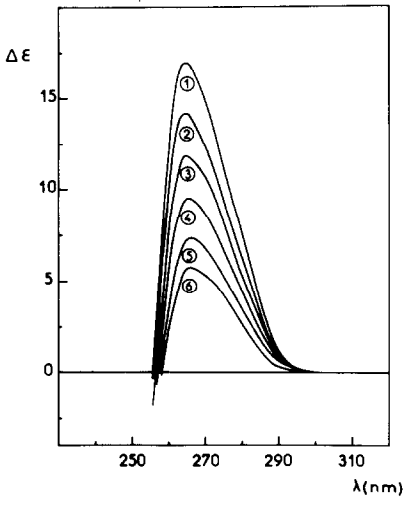

Fig.1. CD spectrum of poly(A) ($2.23.10^{-5}$ M) alone ① and in presence of various concentration of tetrameric *lac* repressor ② $3.1.10^{-7}$ M; ③ $6.2.10^{-7}$ M; ④ $9.3.10^{-7}$ M; ⑤ $1.25.10^{-6}$ M; ⑥ $1.56.10^{-6}$ M.

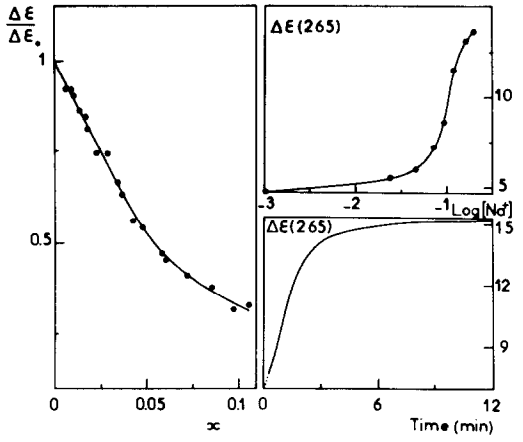

Fig.2. Left: Variation of the CD amplitude of poly(A) upon addition of *lac* repressor; x is the ratio of the *lac* repressor (tetramer) and poly(A) (base) concentrations. Right: (Lower part) effect of trypsin addition on the amplitude of the CD signal poly(A) *lac* repressor complex: C_A $5.66.10^{-5}$ M, C_{Rcp} $3.60.10^{-6}$ M. (Upper part) effect on ionic strength on the intensity of the CD signal of poly(A)–*lac* repressor complex: C_A $5.52.10^{-5}$ M; C_{Rep} $4.46.10^{-6}$ M.

presence of poly(A) at low ionic strength (10^{-3} M phosphate, when the protein is bound) and at 0.2 M phosphate ionic strength (when the complex is dissociated) were compared, and no difference was observed.

We investigated the binding to poly(A) of an i^{-d} mutated *lac* repressor with serine 16 replaced by proline. This repressor which shows modified binding properties to poly[d(AT)] [23], binds to poly(A) exactly as the wild type repressor does.

Addition of small amounts of trypsin to *lac* repressor leads to the formation of a proteolytic core which has lost its binding properties on *lac* operator and on non-specific DNA, but still binds inducers [17–19] and anti-inducers [20]. This core protein lacks about 60 amino acids at the N-terminal part of the molecule. When trypsin is added to *lac* repressor under conditions leading to core formation, as shown by gel electrophoresis, the CD signal of poly(A) increases and in a few minutes reaches the value corresponding to the free polynucleotide.

Addition of the inducer IPTG to the complex poly(A)–repressor does not change the CD signal of poly(A).

4. Discussion

That *lac* repressor binds to poly(A) which is a polyanion is not surprising since it is known that *lac* repressor binds phosphocellulose [14], DNA [4,5] and DNA-cellulose [18,21]. The effect of increasing the ionic strength which reverses the CD change demonstrates that there is a large contribution of electrostatic interactions between the phosphate groups of the poly(A) and the basic residues of the protein. This contribution is also present in the binding of *lac* repressor to the *lac* operator [1] and in the non specific binding to DNA [2–5].

It is particularly remarkable that the binding of *lac* repressor to poly(A) induces a conformational change of the polynucleotide. The change observed in the CD spectrum is similar to that obtained when the temperature is increased which may indicate that the binding leads to an unstacking of adenine bases. A similar effect has been observed when small peptides bearing aromatic residues bind to poly(A) [8]. In these cases there was a stacking of the aromatic aminoacid with the bases of poly(A) leading to a quenching of the quenching of the aromatic amino acid [9].

The fluorescence of *lac* repressor is mainly due to its two tryptophyl residues [16,22]. The absence of fluorescence quenching in the poly(A)–repressor complex shows that neither of them is involved in stacking interactions with adenine bases. However this result does not exclude the participation of tyrosyl residues in the interaction with poly(A) since the quenching of these residues would be difficult to detect and these residues might be quenched prior to complex formation.

From the initial slope of the relative decrease of the CD amplitude as a function of repressor concentration (fig.2), it may be concluded that the binding of one tetrameric repressor to poly(A) leads to the vanishing of the CD signal of about 10 bases. More than 10 bases might of course be involved if their CD signal does not completely vanish. The polynucleotide could be bound at several points on the surface of the protein, and the segments of polymer between these points might be unstacked.

The reversal of the CD change observed when the N-terminal part of the protein is hydrolyzed by trypsin indicates that this part is involved in the interaction with poly(A). However from this experiment it is not possible to conclude if the core protein and small peptides are released from the polynucleotide, or if they are always bound without any effect on its CD spectrum.

It should be noted that the peptides released by the tryptic hydrolysis of *lac* repressor differ with respect to many points from the corresponding sequence in the non-hydrolyzed *lac* repressor: they all bear supplementary positive and negative charge respectively on their N and C terminal residues; their conformation may be changed due to their size, the lack of long range interaction and their new charges; they are not included in a tetrameric structure. All these facts may explain the modification or the lack of interaction of these peptides with poly(A).

5. Conclusion

The above results demonstrate the the *lac* repressor binds to single stranded poly(A) and changes its conformation. As already observed for the specific interaction with the *lac* operator and for the 'non-specific' interaction with DNA this binding is very sensitive to the ionic strength and the N-terminal part of the protein molecule is involved. However differences exist between the non-specific binding on poly[d(AT)] and that on poly(A) as shown by the behaviour of the i^{-d} mutated (Ser 16 → Pro) *lac* repressor.

This work shows that *lac* repressor can bind to a single stranded polynucleotide. Preliminary experiments have shown that the effect of *lac* repressor on the CD spectrum of single stranded poly(C) are smaller than that observed with poly(A). Further work is required to determine whether this is due to a lack of binding or to a reduced effect on the conformation of the polynucleotide. Also this should indicate whether the *lac* repressor binding to polynucleotides involves interactions with bases and could help characterize some of the forces involved in the *lac* repressor–*lac* operator interaction.

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